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Molecular Aspects of the Metabolism and Toxicity of Arsenicals

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Introduction

A remarkable aspect of the metabolism of inorganic arsenic is its conversion to methylated metabolites. Hence, an individual ingesting water containing inorganic arsenic excretes in urine inorganic, methyl, and dimethyl arsenic. Although the formation of methylated arsenicals has been commonly considered a mechanism for detoxification, research in this laboratory shows that intermediates formed in the metabolism of inorganic arsenic are more reactive and toxic. Hence, the methylation of arsenic is an activation process. To gain insight into the mechanistic basis for the methylation of arsenic, we undertook the isolation, purification, and characterization of the enzyme that catalyzes the methylation of arsenic. This research identified a novel *S*-adenosyl-l-methionine-dependent methyltransferase from rat liver cytosol that catalyzes the formation of methylated arsenicals from inorganic arsenic. We have cloned the *cyt19* gene that encodes this enzyme and shown that it is highly conserved in the rat, mouse, and human genomes.

Studies with recombinant rat cyt19 allows us to address two general questions about biomethylation reactions.

- What intermediates and products are formed in the course of methylation reactions catalyzed by cyt19? Are they consistent with those predicted by the widely-accepted scheme for alternate steps of oxidative methylation and reduction of pentavalent arsenic to trivalency as shown in the scheme?

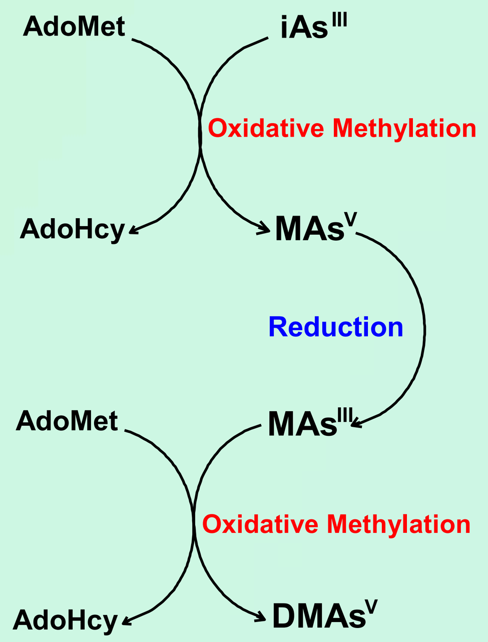
- What are the roles of exogenous and endogenous reductants in support of the catalytic function of cyt19?

Methods

Cloning of rcyt19- The oligonucleotide sequence of rat liver S-adenosyl-L-methionine: arsenic (III) methyltransferase was used to design primers to amplify the gene sequence from a rat liver cDNA library. The full length sequence was inserted into *E.coli* strain DH5α. A clone containing the complete coding sequence of rat *cyt19* was identified and designated pRSET-rcyt19.

Purification of Recombinant Protein- *E.coli* strain BL21(DE3)pLysS was transformed with pRSET-rcyt19. and expression induced by addition of 1mM IPTG to culture media. Recombinant rcyt19 was purified from lysate by affinity chromatography on a Pro-bond Ni-NTA resin column. Purified recombinant rcyt19 was dialyzed against NBB or 100mM TRIS, pH 7.4, 50mM NaCl and stored at -20C.

Methylation activity of recombinant rat cyt19 – The capacity of the enzyme to convert inorganic arsenic (III) to methylated products was monitored in reactions using ^{73}As-labeled arsenic (III) or using pH selective hydride generation-atomic absorption spectrometry to determine the oxidation state of arsenicals.

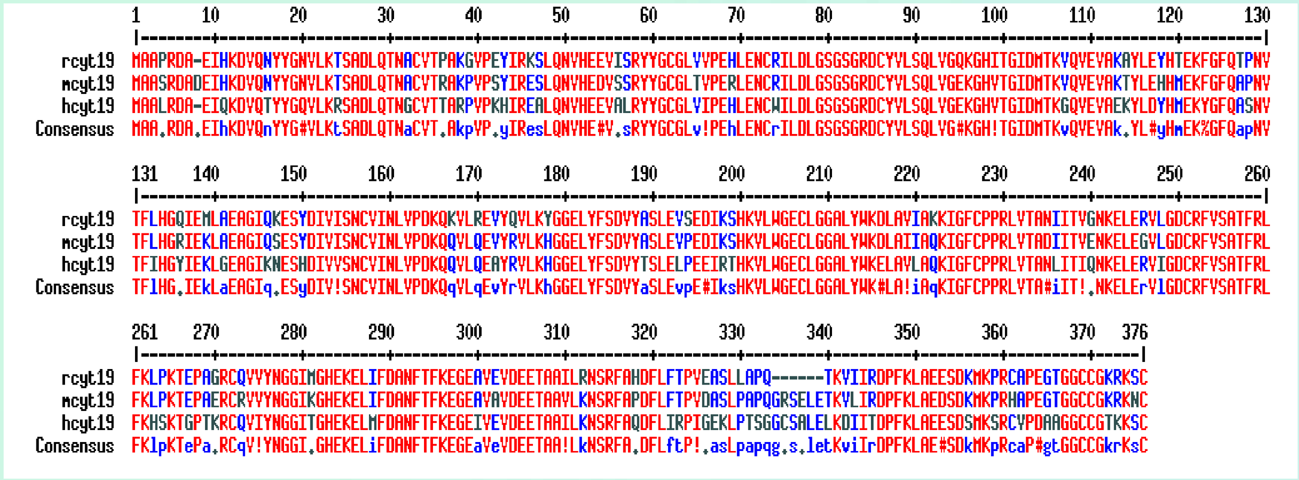


Scheme for the oxidative methylation

and reduction of arsenic

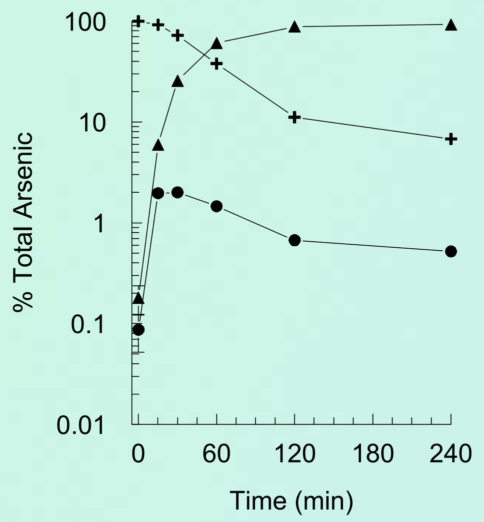
Results

1



Multiple sequence alignment for rat (r), mouse (m), and human (h) cyt19. Consensus sequence for these proteins also shown.

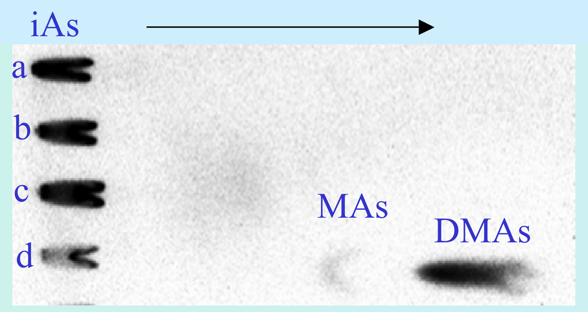
2



Recombinant rat cyt19-catalyzed conversion of inorganic arsenic (+) to methyl (■) and dimethyl (▲) arsenic. Assay conditions: 1 µM inorganic As^{III}, 1 mM S-adenosyl-L-methionine, 1 mM DTT, 100 mM tris/phosphate, pH 7.4, 37C.

3

Thioredoxin (Trx) supports recombinant rat cyt19-catalyzed conversion of inorganic arsenic to methyl and dimethyl arsenic. Lane a – Trx reductase and NADPH, Lane b – Trx and NADPH, Lane c – Trx and Trx reductase, Lane d – Trx, Trx reductase, and NADPH. Assay conditions: Lanes a-c incubated for 16 hours at 37C, lane d for 15 minutes at 37C.



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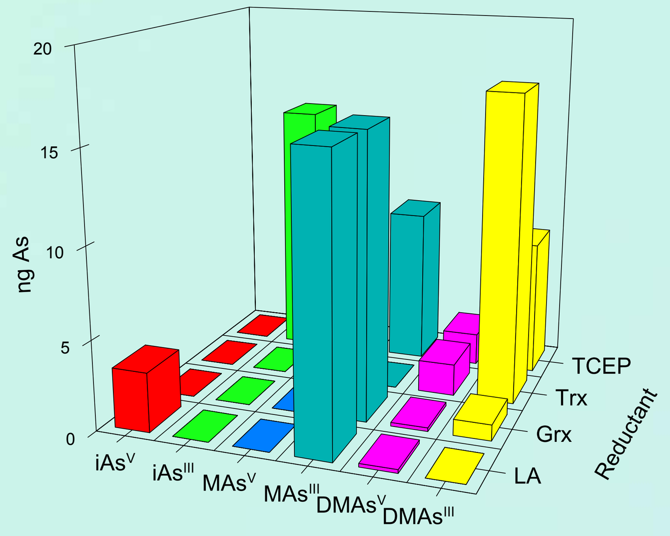

Effect of various reductants on recombinant rat cyt19-catalyzed conversion of inorganic arsenic to methyl and dimethyl arsenicals.
Reductants: TCEP, triscarboxyethylphosphine with glutathione (GSH)
Trx – Thioredoxin with thioredoxin reductase, NADPH, and GSH
Grx – Glutaredoxin with GSH reductase, NADPH, and GSH
LA – Lipoic acid with thioredoxin reductase, NADPH, and GSH
Assay conditions: 1 µM inorganic As^{III}, 1 mM S-adenosyl-L-methionine, 100 mM tris/phosphate, pH 7.4, 37C for 15 minutes.

Conclusions

- The *cyt19* gene in rat, mouse, and human genomes encodes an arsenic methyltransferase. Cloning of these orthologous genes and expression of their recombinant protein products confirms that each catalyzes the conversion of inorganic arsenic to methylated products.

- The catalytic activity of recombinant rat cyt19 depends upon the presence of reductants in the reaction mixture. Three endogenous reductants – thioredoxin, glutaredoxin, and lipoic acid – have been identified. Thioredoxin is the most effective reductant and must be recycled to support the reaction.

- Analysis of intermediates formed in cyt19-catalyzed reactions shows that both pentavalent and trivalent arsenicals are present in the reaction mixture. This is consistent with the postulated scheme for arsenic methylation that involves alternating steps of oxidative methylation and reduction of pentavalent arsenicals.

Impact

- Identifying molecular components of the pathway that converts inorganic arsenic to more reactive and toxic species facilitates studies on the mode of action of arsenic as a toxin and carcinogen.

Future Directions

- Examine the molecular processes involved in arsenic methylation by cyt19, especially the role of reductants in catalysis.

- Investigate relation between genotypic variation in *cyt19* and its phenotype as reflected by its capacity to methylate arsenic and its role in genetic basis for interindividual variation in the capacity to methylate arsenic.

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